Detection of 17 Targets in a Single PCR Tube by a Novel MeltPlex® Probe System Combining Melting Curves and Taqman Probes

Søren M Echwald. Anapa Biotech A/S, Agern Allé 1, 2970 Hørsholm, Denmark.
Email: sme@anapabiotech.com

Introduction

Despite the developments in conventional PCR, the complexity of multiplex Real Time PCR is still limited due to the lack of sufficient detection channels. To achieve high-end multiplexing capacity on standard Real Time PCR machines, Anapa Biotech has developed the MeltPlex® technology (see box on right).

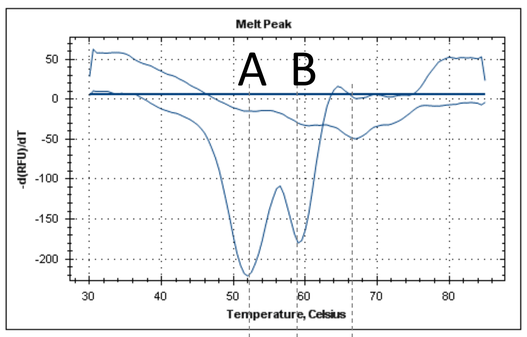
MeltPlex® utilizes a system of labelled Taqman-type probes allowing each to be read out by subsequent melting curve analysis. 5 or more probes can be analyzed per fluorophore channel. By utilizing melting curve readout of modified probes – one for each target - rather than the only amplicons, the system adds an extra level of specificity to melting curve analysis. Reaction and melting analysis is performed without the need to re-open PCR reaction tubes.

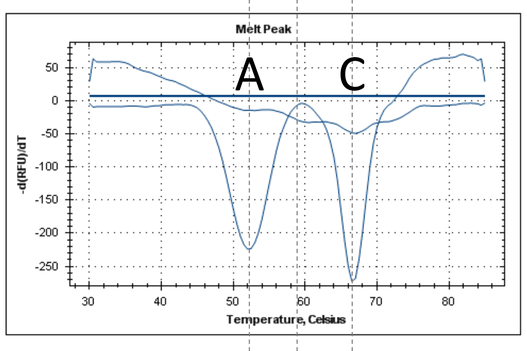
Detection reaction chemistry is similar to TaqMan and MeltPlex® probes can easily be designed using existing TaqMan target sequences

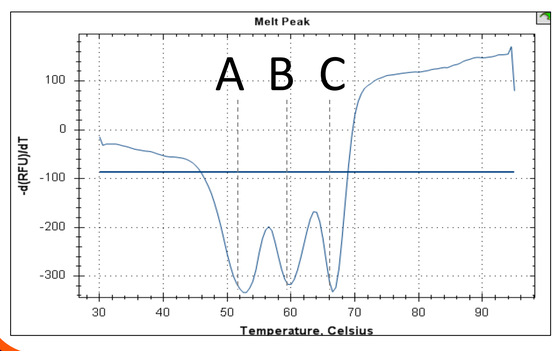
To illustrate the principle, we designed FAM-labeled MeltPlex® probes against 3 artificial targets (A, B and C) with melt-domains at 52°C (A), 60°C (B), and 67°C (C).

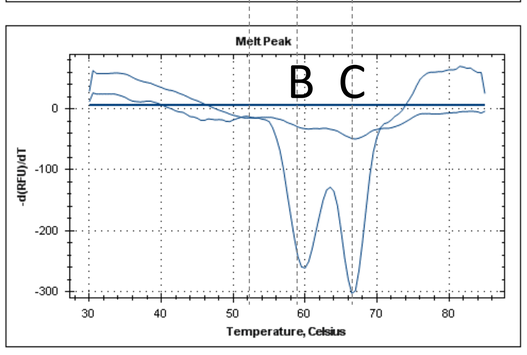
Targets were PCR amplified in the presence of all three detection probes and subjected to a subsequent melting curve analysis.

Below are PCR amplification- and melt-curves of a sample with all targets, and right with all three combinations of target pairs, detected in the same (FAM) channel



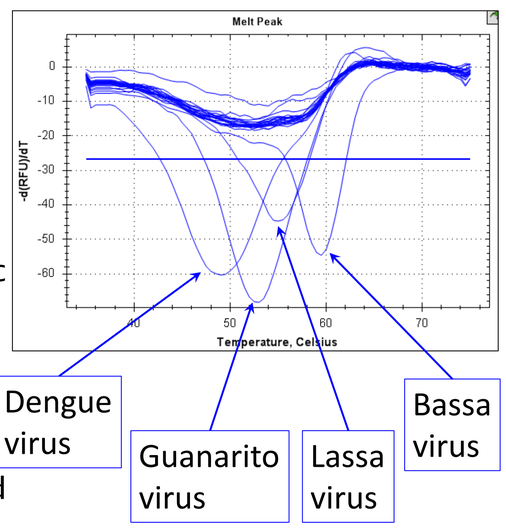


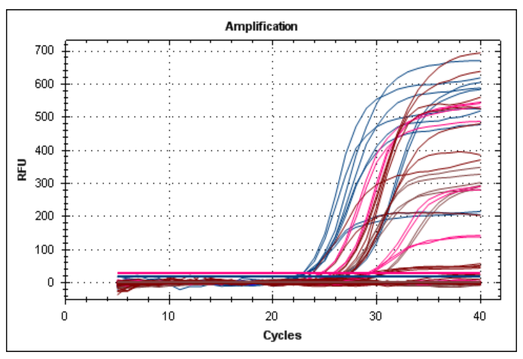




To test the system we designed MeltPlex® probes based on previously designed TaqMan probes targeting 17 different hemorrhagic fever viruses (1) and tested them against artificial DNA targets. Targets were amplified in singleplex in the presence of all 16 MeltPlex probes against the group of viruses. All assays resulted in a specific meltingcurve, which is clearly detectable over the background of all probes.
To the right are shown meltingcurves specific for viruses in the CAL570 and CAL 610 channels. Below the combined QPCR amplification curves.

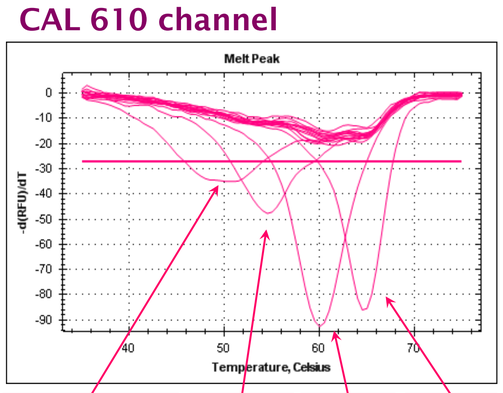
CAL 560 channel





QPCR amplification curves with a mix of 16 MeltPlex probes and a single PCR-primer pair per well.

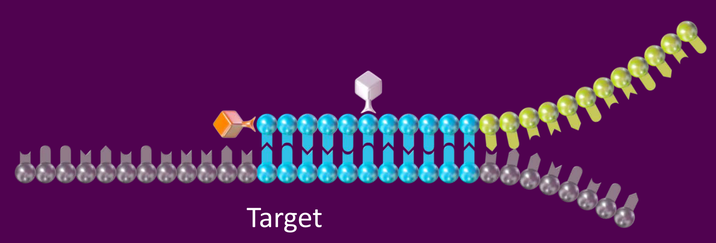
CAL 610 channel



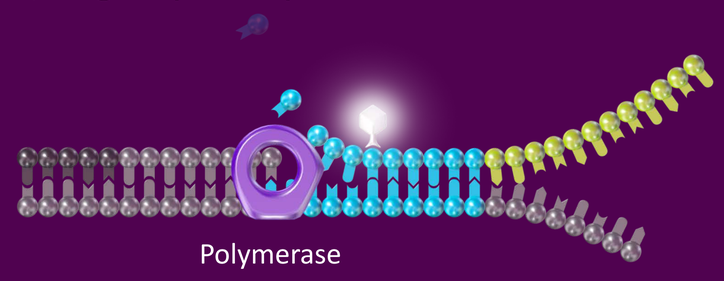
MeltPlex® multiplex PCR process

MeltPlex employs redesigned Taqman-type probes, with an internal fluorophore and a 5’ quencher. In addition, each of the probes has been equipped with a unique melting domain of varying length

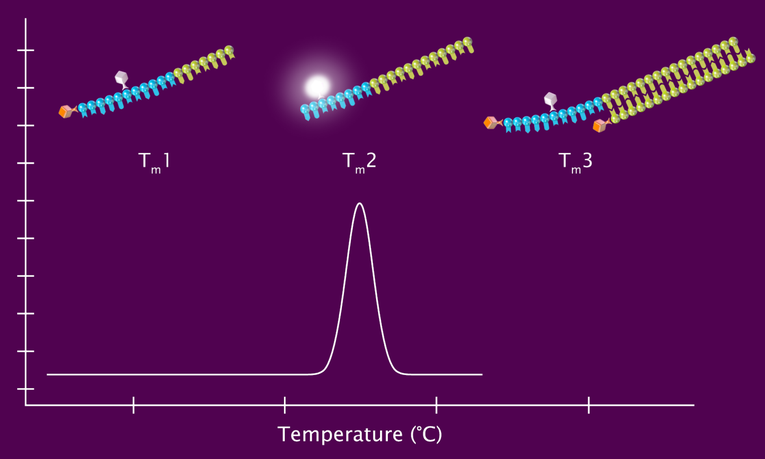
If the probe target is present, the probe binds to the target region,



and the 5’ quencher is released during PCR amplification, leaving an activated, target-specific probe.



Immediately following PCR, a melting curve analysis is performed, where a common quenching probe hybridizes to the melting domain of the target probes



As the quenching probe dissociates, only the activated target probes will provide a melting curve readout, since the fluorophore on the non-activated probes remains quenched.

The melting curve analysis makes it possible to determine exactly which of up to 25 probes have been activated and hence displays the content of target analytes in the sample.

Summary

Using a pre-determined set of probe design rules, 16 existing Taqman-probes designed to detect a range of hemorrhagic viruses were converted to MeltPlex® probes and combined into a single, multiplex, probe-based assay. Using 4 of the 5 channels of the Bio-Rad CFX96 instrument, we have shown proof-of-concept that the MeltPlex® system can reliably detect 17 individual viral sequences.

Conclusion

MeltPlex® comprise a robust, high-multiplex, homogeneous system to provide 20+ readouts per PCR reaction.

(1) Pang, Zheng et al. “Comprehensive Multiplex One-Step Real-Time TaqMan qRT-PCR Assays for Detection and Quantification of Hemorrhagic Fever Viruses.” Ed. Stefan Dübel. *PLoS ONE* 9.4 (2014): e95635. *PMC*. Web. 2 Apr. 2017.